Claim 1 has been rejected under 35 U.S.C. § 101 for directed to non-statutory subject matter. The Examiner states that claimed invention cannot be a product of nature, and suggests amending to recite an "isolated" molecule. Applicants have amended the claim, and thus obviated the rejection. Applicants request the rejection be withdrawn.

Claims 1-5 have been rejected under 35 U.S.C. § 112, first paragraph as claiming subject matter not enabled within the specification. The Examiner contends the specification does not provide enabling disclosure for structural or functional characteristics of fragments comprising amino acids 26-48, 26-49, 26-50, 26-110, 26-114, and 115-139. The Examiner's view is that the sequence for each of the entire polypeptide bands is not named, and therefore, because protein structure prediction is an unpredictable art, it would require undue experimentation to determine the amino acid sequences.

Applicants have canceled claims 4 and 5 which are directed to fragments of zins1 polypeptide and polynucleotides encoding said polypeptides. With regard to claims 1-3, the molecules that are claimed recite a sequence of nucleotides that encode for the mature polypeptide and protein. The specification clearly enables these molecules. As the Examiner points out in the instant Office Action at page 5, sequencing of the isolated polypeptides have amino acid residue 26 at the N-terminus, and the entire sequence is presented in SEQ ID NOS: 1 and 2. Claims 1-3 are product-by-process claims and comprise the expressing a molecule comprising a nucleotide sequence that encodes for the mature polypeptide. The examples in the specification provide the requisite enablement. For example, in addition to providing the sequences, Example 1 discloses that by transfecting a host cell with the polynucleotide molecule of claim 1, a protein was expressed. Example 2 illustrates that the claimed molecule was active. Therefore, Applicants traverse the rejection of claims 1-3, because the claims are fully enabled both within the specification by description and illustration by way of example.

Claims 4 and 5 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. It is the contention of the Examiner that the claim language "first polypeptide" and "second polypeptide" are not clear in the context of being an isolated protein. Applicants have canceled claims 4 and 5, thereby obviating the rejection.

Claims 1-5 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Chassin et al., (Genomics, 29:464-470, 1995). The Examiner contends that Chassin teaches an isolated protein that comprises amino acids 26-48, 26-49, 26-50, 26-110, 26-114, and 115-139. Applicants traverse this rejection in part because claims 1-3 do not recite these fragments, but are directed to a protein that has been produced by expressing a DNA segment comprising a nucleotide sequence as shown in SEQ ID NO: 1 from nucleotide 76 to nucleotide 417. Applicants are uncertain how the Examiner has determined that the cited reference anticipates this molecule. Chassin describes a sequence of nucleotides and amino

acids encoded therefrom that include a signal peptide (numbers –1 to –17), and an intron/exon structure which constitutes the human INSL4 gene. While there is similarity in the overall sequences, the structure of the protein is very different and does not anticipate the present invention. The present invention claims a protein shorter by eight amino acid residues at the N-terminus than the Chassin polypeptide because the secretory signal sequence in the instant application is predicted to be longer ending at residue 25 (Ala), rather than residue 17 (Ser) as predicted by Chassin. No where in either the cited Chassin reference or Appendix A is a mature protein of 114 amino acid residues taught. In fact, Figure 2 of Chassin et al., teaches that based on the authors' structural analysis, the mature polypeptide N-terminus is at residue 18, and while the present inventors expressed, sequenced and identified certain activities associated with claimed protein, the references do not suggest that EPIL was ever even expressed to verify their predictions. Therefore, Applicants respectfully submit that neither the sequence nor reference cited by the Examiner teach a protein that anticipates the claimed invention, and request the rejection be withdrawn and the claims allowed.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned **"Version with markings to show changes made."**

If for any reason the Examiner believes that a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (206) 442-6672.

Respectfully Submitted,

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Registration No. 37,438

Enclosures:
Version with Markings to Show Changes Made
Return Postcard

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

On page 1, at line 5, the title has been amended as follows:

ZINS1 POLYPEPTIDE COMPOSITION STIMULATING PANCREATIC ISLET GROWTH ~~Compositions and Methods for Stimulating Pancreatic Islet Cell Regeneration~~

On page 1, at line 8, a paragraph was inserted as follows:

"REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Patent Application Serial No. 08/991,890, filed December 12, 1997, now U. S. Patent No. 6,114,307, issued on September 5, 2000, which is related to U.S. Provisional Patent Application Serial No. 60/0333,003, filed on December 16, 1996. Under 35 U.S.C. §§ 119(e)(1) and 120, this application claims benefit of said applications and patent."

On page 42, the paragraph starting on line 10 was amended as follows:

The tissue was dehydrated with a graded series of ethyl alcohols, cleared with xylene, and infiltrated with PARAPLAST X-TRA ~~Paraplast X-tra~~ (Fisher Scientific, Pittsburgh, PA) using a TISSUE-TEK ~~Tissue-Tek~~ VIP2000 (Miles, Inc., Elkhart, IL).

On page 42, the paragraph starting on line 14 was amended as follows:

The flattened pancreas was removed from the biopsy bag using forceps and embedded longitudinally with PARAPLAST X-TRA ~~Paraplast X-tra~~. All pancreata were oriented the same way in the block, with the head of the pancreas placed in one corner of the embedding mold, the tail of the pancreas in the opposite corner, and the body in the middle of the mold.

On pages 42-43, the paragraph starting on line 25 of page 42 was amended as follows:

The sections were stained with Harris hematoxylin (Sigma, St. Louis, MO) and Eosin histology staining (Surgipath, Richmond, IL). The number and size of islets per longitudinal section of the pancreas were counted and measured by using a camara-lucida attached to a light microscope (10X objective, Olympus, BH-2), interfaced to a BIOQUANT

SYSTEM IV ~~BioQuant System IV~~ image analysis system (B&M Biometric, Inc., Nashville, TN). After calibration, the electronic pen of the digitizer was used to carefully trace the outline of each islet profile by screening the whole section of the pancreas. Simultaneously, the data was computed and stored. Data analyses were performed by using ANOVA (GraphPad Software, San Diego, CA) followed by unpaired t test.

On page 46, the paragraph starting on line 14 was amended as follows:

A sample of Zins1, purified as described above, was run on a NOVEX ~~Novex~~ 18% Tris-Glycine gel (NOVEX ~~Novex~~, San Diego, CA) under reducing conditions (2-mercaptoethanol). An electroblot transfer to PVDF membrane was performed in 10mM CAPS buffer pH 11.0, 10% methanol at 200mA for 1 hour at 4°C. The PVDF blot was visualized with Coomassie blue staining. Stained protein bands were excised for Edman degradation N-terminal protein sequencing on an Applied Biosystems 476A Protein Sequencer (Foster City, CA) using standard protocols and FSTBLT cycles. The data was analyzed using Applied Biosystems Model 610A Data Analysis System, v.1.2.2).

On pages 46-47, the paragraph starting on line 28 of page 46 was amended as follows:

A Michrom BioResources MAGIC ~~Magic~~ 2002 HPLC system (Michrom BioResources, Inc., Auburn, CA) equipped with a 1.0 x 150 mm Monitor C18 100Å 5m column (Michrom BioResources, Inc.) was used at a flowrate of 50 µl/min and a column temperature of 30°C. Typically, 5.0 µg of whole or digested protein was injected onto the column equilibrated in 5% B and a linear gradient from 5 to 85% B over 80 minutes was immediately initiated (A: 2% acetonitrile + 0.1% acetic acid + 0.020% TFA, B: 90% acetonitrile + 0.1% acetic acid + 0.018% TFA). The outlet from the HPLC UV detector was plumbed directly into a Finnigan LCQ Ion Trap Mass Spectrometer (Thermoquest Corp., San Jose, CA) with no flow splitting, a heated capillary temperature of 220°C, and a sheath gas flow of 75 (arbitrary units). The source voltage was 5.60 kV and the capillary voltage was 41.00 V. Mass spectra from 300-2000 m/z were recorded continuously during the gradient with 3 microscans per full scan. The most intense [M+2H]2+ ion in each spectrum was automatically selected by the LCQ for zoom scan and MSMS at 25% collision energy.

On pages 48-49, the paragraph starting on line 24 of page 48 was amended as follows:

5 µg each of untreated, PNGaseF-treated, and sialidase-treated Zins1 NF was diluted with an equal volume of NOVEX ~~Novex~~ 2X Tris-Glycine SDS sample buffer (NOVEX ~~Novex~~, San Diego, CA), boiled for 3-5 minutes, and loaded onto a NOVEX ~~Novex~~ 18% Tris-Glycine gel. In addition, 5 µg each of untreated, PNGaseF-treated, and sialidase-treated Zins1 NF was diluted with an equal volume of NOVEX ~~Novex~~ 2X Tris-Glycine SDS sample buffer (NOVEX ~~Novex~~, San Diego, CA) containing 5% b-mercaptoethanol, boiled for 3-5 minutes, and loaded onto a NOVEX ~~Novex~~ 18% Tris-Glycine gel. Both the non-reduced and reduced gels were run at a constant voltage of 125V and visualized with Coomassie Blue staining. NOVEX ~~Novex~~ Mark 12 Wide Range Protein Standards were used to determine apparent molecular weights.

On page 49, the paragraph starting on line 17 was amended as follows:

Confirmation of the putative O-glycosylation was obtained via monosaccharide composition analysis. Monosaccharide composition for Zins1 was analyzed as follows: Monosaccharide composition was carried out on a Dionex system composed of a DX500 HPLC with an ED40 electrochemical detector, a GP40 pump, and a CARBOPAC-PA ~~CarboPac PA~~ 10 column (Dionex, Sunnydale, CA). In both types of analyses, Dionex monosaccharide standards were used to calibrate the instrument. The glycoprotein fetuin was used as a positive control (Sigma, St. Louis, MO).

On page 55, the paragraph starting on line 1 was amended as follows:

Conditioned culture medium removed from these islet cells was added to cultures of normal BALB/c islets were isolated in MATRIGEL ~~Matrigel~~ Basement Membrane Matrix (Collaborative Biomedical Products, Bedford, MA). The normal mouse islet phenotype changed, becoming huge with much branching and forming cyst-like structures. This conditioned medium was designated IDC53.1. Various other conditioned media obtained either from cultures of osteoclast, osteoblast or dendritic cells obtained from p53-/- knockout mice (see WO 9607733), or from cultures of normal C57/Black 6 islet cells, did not exhibit this activity. In addition, normal BALB/c islets placed in this conditioned medium developed "cobblestone" cells all around the islet. This effect was not seen when various other conditioned media were tested.